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EDITORIAL

Sex matters

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In 2015, the NIH required that all grants must consider sex as a biological variable (SABV) in their experimental plan. Not only were you required to consider SABV, but also to provide expected outcomes and relevant statistical analyses to test for potential sex differences [1, 2]. Yes, the time had come to shine a light on the importance of SABV. But let's back up for a minute and review why SABV had been shelved for so long for most labs—when and how did the male become the control group? As reviewed by Beery and Zucker, studies across neuroscience by 2009 had focused on male only outcomes 5.5 times more often than those that included females, and this number was unchanged from the previous 50 years [3]. While it is unclear as to the specific reasons why the field of neuroscience became fearful of using females, the general assumption that females are somehow more 'variable' than males is a clear factor. While this myth has been put to rest by published meta-analyses in mice, rats and humans clearly showing that in many cases *males* are actually *more* variable in outcome measures relevant to neuropharmacology [3–6], yet the fear lives on.

Based on published studies, somewhere in the late 60's and early 70's neuroscientists dramatically shifted their approach away from being sex inclusive, or at the very least, sex agnostic, to a significant male-bias [7]. In 1969, 20% of studies using animal models reported examining males only, but by 1979 this number had jumped to 70%. Studies focusing on females or inclusive of both sexes had fallen to less than 10%. It is not clear if this dramatic shift over a decade was produced by an increased awareness by the field for a possible role of gonadal hormones in relevant measures thus perpetuating the fear of variability, or if around this time journals began requiring authors to declare the sex of the species they were using, and thus it became more apparent as to the prevalence in neuroscience research in which the male was now the standard control subject.

What has become clear in the last decade is the firm appreciation for SABV in preclinical and clinical research as essential for our understanding of factors contributing to disease risk and resilience. Studies focusing on examining sex differences have demonstrated across the lifespan, from development to maturation and aging, that males and females can differ significantly. From sex chromosomes that allow for disparities in gene dosage and regulatory mechanisms, to the important role of gonadal hormones, our appreciation for the unique and highly mechanistic insight that including SABV affords us has gained great attention. This special issue provides coverage across areas most relevant to the Neuropsychopharmacology audience of the current state of SABV and where sex differences have undoubtedly demonstrated valuable insight. This is by no means a comprehensive review of all research in these areas, but rather a selection of appropriate and representative examples to provide guidance and reference for the inclusion of SABV as an essential component of research in Neuropsychopharmacology.

Critical to emphasizing the importance of SABV inclusion is the warning labels all too common in the pharmaceutical industry for the very real consequences of a previous failure to include both sexes in clinical trials. Despite knowing that females are at greater risk for a wide-range of adverse drug reactions, limited inclusion of women in randomized clinical trials has occurred in the recent past [8–10]. Results from the Physicians' Health Study that included over 20,000 *male* physicians in the 1980's suggested that low-dose aspirin was associated with a reduced risk of myocardial infarction (1989). However, decades later a follow-up study examining 39,000 women showed surprising outcomes in which low dose aspirin produced a greater risk of stroke and no overall effect on myocardial infarction risk in women [11]. In addition, the FDA recently approved labeling changes for dosing of the sleep drug, zolpidem (Ambien). The recommended starting dose was significantly reduced in women where the drug had a significantly slower rate of metabolism, resulting in higher drug concentrations and significant lasting effects not seen in men. To this point, of the ten prescription drugs removed from the U.S. market between 1997 and 2001, eight had greater adverse effects in women compared to men [12].

As to the biology and where sex differences originate, all cells have a *sex*, designated by the presence and dosage of X or Y chromosomes, which in most cases will be XX (female) or XY (male). However, *gender* is a designated societal determinant and traditionally described only in humans [13]. Importantly, the sexually dimorphic brain, similar to most sex differences, does not fall into a binary categorization—but rather is on a continuum or spectrum with each cell and each brain region comprised of varying degrees of 'maleness' and 'femaleness' based on an average report within each sex [14, 15]. Finally, the combination of genetic sex and gonadal hormones can promote dramatic changes in trajectory during important periods of brain development and maturation.

In this special issue, Green et al. begin at the most basic biological point of deciphering sex differences—the DNA [16]. The authors examine the rates and symptom severity of neurodevelopmental and neuropsychiatric diseases in typically developing males (XY) and females (XX) and compare these with sex chromosome aneuploidies (SCA) (e.g., Turner syndrome (X0) and Klinefelter syndrome (XXY)) [16]. This review also discusses the consistent morphological and developmental differences in the SCA brain and their potential relationship with disease symptoms. What is clear from this intriguing discussion, is the vital importance of how *many* X or Y chromosomes each cell has, and that a loss (monosomy) of a sex chromosome is far more detrimental than a trisomy (XXY, XYY or XXX). Building on these chromosomal contributions to sex differences in brain structure and function, in an intriguing review and likely one of the first to tackle a timely and important topic [17], Nguyen et al. discuss how known differences in the sexually dimorphic brain and the role of gonadal hormones inform decisions and outcomes for the transgender brain. This is an intriguing and timely topic, and one that should be read by all clinicians and researchers interested in and working with diverse populations to appreciate the complexities of the sex and gender.

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The next few reviews in this issue tackle the importance of developmental windows in how sex differences in the brain may arise, and how they impact brain function and disease risk across the lifespan. McCarthy, the world expert on sex differences in cellular processes key in brain development, reviews the role of microglia and the inflammatory signaling molecules responding to steroid hormones in shaping the sexually dimorphic brain [18]. This review postulates that an increased risk for males for gestational perturbations and neurodevelopmental disorders may lie in an intersection of maleness with inflammatory processes involved in masculinization. Building on development of the sexually dimorphic brain and the influence of gonadal hormones, Heck and Handa examine the development of sex differences in the hypothalamic-pituitary-adrenal stress axis [19]. Connecting these mechanistic components of immune activation with stress experience during gestation, Goldstein et al. then discuss the programming of sex differences in major depressive disorder and its comorbidity with cardio-metabolic disorders that again supports a contribution across the lifespan for disease risk and resilience that begins in these very early periods of development [20].

Childhood and the adolescent window of brain maturation are critical times for appropriate wiring unique to the limbic system, especially that of the prefrontal cortex, and for promotion of executive functioning and emotional regulation. Two reviews have examined the importance of sex differences here. Kaczkurkin et al. review the evidence for sex differences in brain structure, white matter organization, and perfusion during these developmental periods, and use sex differences to discuss psychopathology, focusing on the presentation of affective disturbance, psychosis, and ADHD [21]. Focusing on executive functioning tasks in humans and animal studies, Grissom and Reyes discuss the paucity of studies that have adequately examined sex differences in this area, and how potential developmental trajectory differences for males and females may confound studies examining attention, focus, and impulse control [22]. An additional behavioral measure that has become a key feature in preclinical models for neurodevelopmental disorders, especially for autism studies, is social behavior. Borland et al. review the current state of sex differences in the presentation and development of social behaviors in humans and animal models, and discuss the potential therapeutic efficacy of oxytocin in social reward and treatment of social dysfunction [23].

Affective disorders in general have minimal sex differences in overall presentation numbers prior to puberty, but during this hormonally dynamic period significant differences dramatically emerge. Females present with an increased risk for affective disturbance beginning in adolescence, and this sex difference continues into adulthood across the remainder of the lifespan. Three reviews discuss potential points of where these sex differences may arise. In the review by Rubinow and Schmidt, the authors build a comprehensive framework for how sex differences in disease risk involve both hormonal and chromosomal mechanisms [24]. A masterful discussion intertwines how dynamic neuroplasticity involved in key neural networks interacts with genetic variants to provide risk or resilience to affective disorders, such as depression and anxiety. Where sex differences arise in these arousal circuits is a focus of the review by Bangasser et al., who discuss important factors common to preclinical animal models and clinical research studies, largely examining stress regulation by corticotropin-releasing factor [25]. Tackling sex differences in affective disorder risk from a therapeutic angle, LeGates et al. examine key factors underlying synaptic transmission and neuronal plasticity that may determine antidepressant drug efficacy [26].

In addition to affective disorders, dramatic sex differences across pain and addiction neurocircuits have contributed to disparities in therapeutic drug treatment and addiction outcomes. Averitt et al. discuss significant differences in pain pathways, pain tolerance, and drug receptor pharmacology, all factors pertinent and vital to solving the current opioid crisis [27]. Examining reward circuits important to addiction, Becker and Chartoff build a conceptual blueprint detailing all levels, from the molecular and epigenetic, to the physiological and behavioral, that underlie sex differences in disease and substance abuse risk [28].

Finally, as we progress across the lifespan in this special issue, processes involved in aging become significant additional factors in neuropsychiatric disease, where women are at greater risk for dementia, and during the window of dynamic hormonal changes of the peripubertal transition women again present with a greater risk for affective disorders and schizophrenia [29]. One of the major peripheral systems thought to be contributing to this disparity is the immune system. Rainville and Hodes discuss the importance of known sex differences in the immune system where females typically have a more reactive immune response, and examine how this may contribute to neural dysfunction, especially when added to an already taxed aging brain. One of the major mammalian brain structures contributing to dramatic sex differences in aging is the hippocampus [30]. The hippocampus is vital to cognitive function, including learning, flexibility, and pattern separation. In our last review of this special issue, Yagi and Galea discuss the layers of known sex differences in hippocampal plasticity and neurogenesis, and how an appreciation of these differences may provide greater insight into mechanisms related to flexibility and aging [31].

While these timely reviews demonstrate valuable insight into the importance of examining sex differences spanning the lifespan, additional work is needed. As our environment becomes ever increasingly complex, understanding how sex differences impact disease risk and resilience will only become more critical. For example, there is a growing need for understanding sex differences in how social media is utilized and its effects on the developing and maturing brain, from fragmented parental care to adolescent screen addiction, all of which impact mental health [32, 33]. Importantly, this special issue highlights the causal and mechanistic value in cases where significant sex differences are found, as well as when they're not; where sex differences may arise at one period of life and disappear in another. Lastly, this special issue reinforces the message that males are not the control, and females are not more variable. The value is in appreciating the difference. Sex matters.

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